

DRAFT 24 August 2005



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION,
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Requests for Waivers from Toxicity and Pathogenicity Testing on Non-target Organisms (Avian Oral, Avian Inhalation, Wild Mammals, Freshwater Fish, Aquatic Invertebrates, Terrestrial and Aquatic Plants, Insects and Honeybees, and Estuarine and Marine Invertebrates) for the Microbial Pesticide (TGAI), *Verticillium* Isolate 850 (EPA Reg. No.71927-R; DP Barcode: 314718; Decision #: 220293. MRIDs 46481101. Submitted by Arcadis, Inc., and IR-4, USDA.

FROM: Gail Tomimatsu, Ph.D.
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division, 7511C

TO: Sharlene Matten, Ph.D.
Regulatory Action Leader
Biopesticides and Pollution Prevention Division, 7511C

CLASSIFICATION: Acceptable rationale for waiving all non-target organism testing. Further toxicity or pathogenicity testing on non-target organisms and environmental fate studies are not justified at this time.

BPPD SUMMARY AND CONCLUSIONS: The rationale and justification to waive non-target avian, terrestrial wild mammals, plants, freshwater fish and aquatic invertebrates, estuarine and marine animals, honeybee and non-target insect testing as submitted by USDA-IR4 on behalf of Arcadis, Inc. are acceptable for the intended uses of *Verticillium* Isolate 850 (DutchTrig™) in susceptible elm species in US landscapes. The new information and data regarding product identity and characterization, as requested in a previous memorandum [Letter of Deficiencies,

DRAFT 24 August 2005

from Phil Hutton (OPP/BPPD) to Dr. Michael Braverman (USDA/IR4) dated 12-06-2002] support a risk characterization and a conclusion of a reasonable certainty that there are no incremental hazards to non-target organisms as a result of intended uses of *Verticillium* Isolate 850.

The requests were based on a general rationale pertaining to the biology and ecology of the MPCA and the Dutch elm disease complex from the open literature, data from host range studies, and the lack of exposures to non-target organisms because of the directed application method (via closed injection of the MPCA into a living tree). *Verticillium* spp. are widely present in soil, as a common inhabitant of crop debris; and only two species of the genus are regarded as plant pathogens. More importantly, once applied, *Verticillium* Isolate 850 appears to be restricted to the annual ring of the elm, and cannot be isolated from elm trees one year after treatment (MRID 457207-04).

From a regulatory perspective, the MPCA will be labeled for use by Professional Arborists or Applicators; all production, distribution, application and disposal of *Verticillium* Isolate 850 are under the guidance and direction of Arcadis, Inc. The pesticide, Dutch Trig™ has been used in the Netherlands since 1992, and in the U.S. since 1999 under an EUP. No adverse effects were observed in workers nor to the environment.

Detailed rationale and justification are specified and appended to the memorandum.

STUDY TYPE: Avian Oral Toxicity/Pathogenicity, Tier I, USEPA OPPTS 885.4050

ACTIVE INGREDIENTS: *Verticillium* Isolate 850

SYNONYMS: Dutch Trig™, *Verticillium albo-atrum* WCS 850, *Verticillium dahliae* WCS 850

REQUESTED BY: ARCADIS, Inc. and USDA/IR-4

WAIVER REQUEST JUSTIFICATION:

The waiver request is based on the following rationale:

SUMMARY: The request to waive avian oral toxicity/pathogenicity studies (OPPTS 885.4050) is based on the rationale that birds will not be exposed to the MPCA, since intended applications are restricted to injections into elm trees. Avian infectivity is unlikely to occur because optimal temperatures for growth and survival are lower (optimal temperature range = 23 to 25°C); than avian body temperatures (it does not grow above 30 °C).

1. The intended application method of direct injection of *Verticillium* Isolate 850 involves a special injector apparatus ("gouge pistol"), a closed system in which the *Verticillium* conidia (spores) are injected into the trunk of an elm tree (3 drops every 3 or 4 inches around the tree trunk's circumference). The *Verticillium* spores are contained in a small glass vial, which is never opened; the vials are "loaded" into the pistol (MRID 457207-04). Because of the "under pressure" in a tree's xylem tissue, the conidia (suspended in water) are sucked up immediately. Used vials, sealed by a septum, are capped and returned to ARCADIS. The injected MPCA is

DRAFT 24 August 2005

contained in the annual ring (MRID # 457207-04, p 19 and 45); furthermore, vascular infection appears contained in treated trees (p. 19 and 45, MRID # 464613-03).

2. The MPCA, *Verticillium* Isolate 850 grows at 23 °C, however is incapable of growing at 30° C (MRID #464613-02).

CLASSIFICATION: The rationale is acceptable to waive non-target avian oral toxicity/pathogenicity studies.

NO REFERENCES FROM THE OPEN LITERATURE CITED

STUDY TYPE: Avian Inhalation Toxicity/Pathogenicity, Tier I, USEPA OPPTS 885.4100

ACTIVE INGREDIENTS: *Verticillium* Isolate 850

SYNONYMS: Dutch Trig™, *Verticillium albo-atrum* WCS 850, *Verticillium dahliae* WCS 850

REQUESTED BY: Arcadis, Inc. and USDA/IR-4

SUMMARY: The request to waive avian inhalation toxicity/pathogenicity studies (OPPTS 885.4100) is based on the rationale that birds will not be exposed to the MPCA, since intended applications are restricted to injections into elm trees. Avian infectivity is unlikely to occur because optimal temperatures for growth and survival are lower than avian body temperatures.

WAIVER REQUEST JUSTIFICATION:

The waiver request is based on the following rationale:

1. The intended application method of direct injection of *Verticillium* Isolate 850 involves a special injector apparatus ("gouge pistol"), a closed system in which the *Verticillium* conidia (spores) are injected into the trunk of an elm tree (3 drops every 3 or 4 inches around the tree trunk's circumference). The *Verticillium* spores are contained in a small glass vial, which is never opened; the vials are "loaded" into the pistol (MRID 457207-04). Because of the "under pressure" in a tree's xylem tissue, the conidia (suspended in water) are sucked up immediately. Used vials, sealed by a septum, are capped and returned to ARCADIS. The injected MPCA is contained in the annual ring (MRID # 457207-04, p 19 and 45); furthermore, vascular infection appears contained in treated trees (p. 19 and 45, MRID # 464613-03).

2. The MPCA, *Verticillium* Isolate 850 grows at 23 °C, however is incapable of growing at 30° C (MRID #464613-02).

CLASSIFICATION: The rationale is acceptable to waive non-target avian inhalation toxicity/pathogenicity studies.

NO REFERENCES FROM THE OPEN LITERATURE CITED

STUDY TYPE: Wild Mammal Testing Tier I, USEPA OPPTS 885.4150

ACTIVE INGREDIENTS: *Verticillium* Isolate 850

DRAFT 24 August 2005

SYNONYMS: Dutch Trig™, *Verticillium albo-atrum* WCS 850, *Verticillium dahliae* WCS 850
REQUESTED BY: Arcadis, Inc. and USDA/IR-4

SUMMARY: The request to waive wild mammal toxicity/pathogenicity studies (OPPTS 885.4150) is based on the rationale that wild mammals will not be exposed to the MPCA, since intended applications are restricted to injections into elm trees. Infection of wild mammal species is unlikely to occur because optimal temperatures for growth and survival are lower than mammalian body temperatures.

WAIVER REQUEST JUSTIFICATION:

The waiver request is based on the following rationale:

1. The intended application method of direct injection of *Verticillium* Isolate 850 involves a special injector apparatus ("gouge pistol"), a closed system in which the *Verticillium* conidia (spores) are injected into the trunk of an elm tree (3 drops every 3 or 4 inches around the tree trunk's circumference). The *Verticillium* spores are contained in a small glass vial, which is never opened; the vials are "loaded" into the pistol (MRID 457207-04). Because of the "under pressure" in a tree's xylem tissue, the conidia (suspended in water) are sucked up immediately. Used vials, sealed by a septum, are capped and returned to ARCADIS. The injected MPCA is contained in the annual ring (MRID # 457207-04, p 19 and 45); furthermore, vascular infection appears contained in treated trees (p. 19 and 45, MRID # 464613-03).
2. The MPCA, *Verticillium* Isolate 850 grows at 23 °C, however is incapable of growing at 30° C (MRID #464613-02).

CLASSIFICATION: The rationale is acceptable to waive non-target wild mammal toxicity/pathogenicity studies.

NO REFERENCES FROM THE OPEN LITERATURE CITED

STUDY TYPE: Freshwater Fish Toxicity/Pathogenicity, USEPA OPPTS 885.4200 (MRIDs 45720704; 46461303)

ACTIVE INGREDIENTS: *Verticillium* Isolate 850

SYNONYMS: Dutch Trig™, *Verticillium albo-atrum* WCS 850, *Verticillium dahliae* WCS 850

REQUESTED BY: Arcadis, Inc. and USDA/IR-4

SUMMARY: The request to waive freshwater fish toxicity/pathogenicity studies (OPPTS 885.4200) is based on the rationale that freshwater fish will not be exposed to the MPCA, since intended applications are restricted to injections into elm trees. Furthermore, once applied, *Verticillium* appears to be restricted to the annual ring of the elm, and cannot be isolated from elm trees one year after treatment (MRID 457207-04).

WAIVER REQUEST JUSTIFICATION:

The waiver request is based on the following rationale:

DRAFT 24 August 2005

1. The intended application method of direct injection of *Verticillium* Isolate 850 involves a special injector apparatus ("gouge pistol"), a closed system in which the *Verticillium* conidia (spores) are injected into the trunk of an elm tree (3 drops every 3 or 4 inches around the tree trunk's circumference). The *Verticillium* spores are contained in a small glass vial, which is never opened; the vials are "loaded" into the pistol (MRID 457207-04). Because of the "under pressure" in a tree's xylem tissue, the conidia (suspended in water) are sucked up immediately. Used vials, sealed by a septum, are capped and returned to ARCADIS. The injected MPCA is contained in the annual ring (MRID # 457207-04, p 19 and 45); furthermore, vascular infection appears contained in treated trees (p. 19 and 45, MRID # 464613-03).

CLASSIFICATION: The rationale is acceptable to waive non-target freshwater fish toxicity/pathogenicity studies.

NO REFERENCES FROM THE OPEN LITERATURE CITED

STUDY TYPE: Aquatic Invertebrate Acute Toxicity/Pathogenicity, USEPA OPPTS 885.4240 (MRIDs 45720704; 46461303)

ACTIVE INGREDIENTS: *Verticillium* Isolate 850

SYNONYMS: Dutch Trig™, *Verticillium albo-atrum* WCS 850, *Verticillium dahliae* WCS 850

REQUESTED BY: Arcadis, Inc. and USDA/IR-4

SUMMARY: The request to waive aquatic invertebrate toxicity/pathogenicity studies (OPPTS 885.4240) is based on the rationale that aquatic invertebrates will not be exposed to the MPCA, since intended applications are restricted to injections into elm trees. Furthermore, once applied, *Verticillium* appears to be restricted to the annual ring of the elm, and cannot be isolated from elm trees one year after treatment (MRID 457207-04).

WAIVER REQUEST JUSTIFICATION:

The waiver request is based on the following rationale:

1. The intended application method of direct injection of *Verticillium* Isolate 850 involves a special injector apparatus ("gouge pistol"), a closed system in which the *Verticillium* conidia (spores) are injected into the trunk of an elm tree (3 drops every 3 or 4 inches around the tree trunk's circumference). The *Verticillium* spores are contained in a small glass vial, which is never opened; the vials are "loaded" into the pistol (MRID 457207-04). Because of the "under pressure" in a tree's xylem tissue, the conidia (suspended in water) are sucked up immediately. Used vials, sealed by a septum, are capped and returned to ARCADIS. The injected MPCA is contained in the annual ring (MRID # 457207-04, p 19 and 45); furthermore, vascular infection appears contained in treated trees (p. 19 and 45, MRID # 464613-03).

CLASSIFICATION: The rationale is acceptable to waive non-target aquatic invertebrate toxicity/pathogenicity studies.

DRAFT 24 August 2005

NO REFERENCES FROM THE OPEN LITERATURE CITED

STUDY TYPE: Estuarine/Marine Organisms Toxicity/Pathogenicity, USEPA OPPTS 885.4280 (MRIDs 45720704; 46461303)

ACTIVE INGREDIENTS: *Verticillium* Isolate 850

SYNONYMS: Dutch Trig™; *Verticillium albo-atrum* WCS 850, *Verticillium dahliae* WCS 850

REQUESTED BY: Arcadis, Inc. and USDA/IR-4

SUMMARY: The request to waive estuarine/marine organisms toxicity/pathogenicity studies (OPPTS 885.4280) is based on the rationale that these organisms will not be exposed to the MPCA, since intended applications are restricted to injections into elm trees. Furthermore, once applied, *Verticillium* appears to be restricted to the annual ring of the elm, and cannot be isolated from elm trees one year after treatment (MRID 457207-04).

WAIVER REQUEST JUSTIFICATION:

The waiver request is based on the following rationale:

1. The intended application method of direct injection of *Verticillium* Isolate 850 involves a special injector apparatus ("gouge pistol"), a closed system in which the *Verticillium* conidia (spores) are injected into the trunk of an elm tree (3 drops every 3 or 4 inches around the tree trunk's circumference). The *Verticillium* spores are contained in a small glass vial, which is never opened; the vials are "loaded" into the pistol (MRID 457207-04). Because of the "under pressure" in a tree's xylem tissue, the conidia (suspended in water) are sucked up immediately. Used vials, sealed by a septum, are capped and returned to ARCADIS. The injected MPCA is contained in the annual ring (MRID # 457207-04, p 19 and 45); furthermore, vascular infection appears contained in treated trees (p. 19 and 45, MRID # 464613-03).

CLASSIFICATION: The rationale is acceptable to waive non-target estuarine/marine organism toxicity/pathogenicity studies.

NO REFERENCES FROM THE OPEN LITERATURE CITED

STUDY TYPE: Non-target Plant Studies, USEPA OPPTS 885.4300 (MRIDs 45720704; 46481101)

ACTIVE INGREDIENTS: *Verticillium* Isolate 850

SYNONYMS: Dutch Trig™, *Verticillium albo-atrum* WCS 850, *Verticillium dahliae* WCS 850

REQUESTED BY: Arcadis, Inc. and USDA/IR-4

SUMMARY: The request to waive non-target plant toxicity/pathogenicity studies (OPPTS 885.4150) is based on the rationale of the directed application method, and a lack of potential for inoculum (of the microbial a.i.) survival and proliferation outside the intended use environment. The *Verticillium* spores are contained in a small glass vial, which is never opened; the vials are "loaded" into the pistol (MRID 457207-04), and the spore suspensions are sucked up immediately, due to the "under pressure" in a tree's xylem tissue. Used vials, sealed by a

DRAFT 24 August 2005

septum, are capped and returned to ARCADIS, Inc. Once applied, *Verticillium* appears to be restricted to the annual ring of the elm, and cannot be isolated from elm trees one year after treatment (MRID 457207-04).

The active ingredient was formerly characterized/classified as *Verticillium dahliae* isolate WCS 850 (Registration No.71927-R; PC Code 081305) in previous submissions. Under certain environmental conditions *Verticillium dahliae* readily produce microsclerotia, which are small black overwintering/resting structures, and may also be infective; also few isolates of *V. albo-atrum* produce microsclerotia. Both species have extensive plant host ranges. Dutch Trig™ (*Verticillium* WCS 850) is a "hyaline mutant" that has not produced microsclerotia since laboratory production in the 1980s; and data provided in MRID 46481101 (present submission) verify the lack of microsclerotia production using acceptable plant pathology techniques.

WAIVER REQUEST JUSTIFICATION:

The waiver request is based on the following rationale:

- 1. Non target plants are not exposed to *Verticillium* Isolate 850, because of the closed delivery system of direct injection.** Sealed vials which contain the active ingredient (10^7 conidia /ml) is transported to the area (of targeted elm trees) at 4 °C. Vials are clicked into a special injector apparatus (gouge pistol), which injects the active ingredient directly into the trunk of an elm tree in early spring. The "under pressure" in the xylem aides in immediate suction of the conidia suspension. Since the vials are sealed, and the contents are delivered in a "closed" gouge pistol, there is virtually no chance of spillage. The injected fungus (vascular infection) is contained in the annual ring (MRID #s 45720704, 46481101).
- 2. *Verticillium* Isolate 850 cannot be isolated from treated elm trees.** As reported in MRID #46481101, studies performed by the manufacturer showed that *Verticillium* could not be isolated 1 meter above the inoculation points from elm tr ees after treatment.
- 3. Lack of survival structures and lack of concerns for increasing inoculum density/potential at treated sites, and practical avirulence of the MPCA.** *Verticillium* Isolate 850 does not produce microsclerotia at ambient or at 72° F; and is thought to be a hyaline (colorless) mutant of the plant-pathogenic *Verticillium albo-atrum* or of *V. dahliae*, both of which produced black resting structures *in vitro*. In laboratory studies, two isolates of *V. dahliae* from rose and okra produced microsclerotia when grown on two agar media (corn meal and Czapek-Dox which promote microsclerotia development); yet no microsclerotia were noted from *Verticillium* Isolate 850 (MRID 4641302 and 46481101). Also, no disease symptoms developed in susceptible trees [19 species including 3 *Ulmus* (elm) species] when challenged with inoculum of DutchTrig™, because of the low virulence of *Verticillium* Isolate 850 (MRID 45720704). *Verticillium* Isolate WCS 850 could not be re-isolated from elm tree cores of the injection site(s), at 2, 4 and 6 weeks after treatment, indicating the ephemeral nature of the fungal active ingredient (MRID 45720704). Furthermore, the MPCA appears to be an avirulent mutant (=practically avirulent) of either *Verticillium dahliae* or *V. albo-atrum*, incapable of inciting disease in *Verticillium*-susceptible plant hosts (MRID 46461303).

DRAFT 24 August 2005

4. Directions for use and and precautionary label language preclude exposures to non-target plants and endangered plant species. The use directions include targeted and directed application (injection) via a closed system to elm trees in an urban landscape, one time per year (usually in the spring by a certified applicator). Accordingly, intended exposures to non-target plants, including endangered/threatened plant species are virtually non-existent. Furthermore, precautionary label language also states "Avoid treating in the proximity of crops susceptible to *Verticillium* without the consent of the grower due to unforeseen possibility that treated trees may produce infectious inoculum."

BIBLIOGRAPHY:

Commonwealth Mycological Institute(C.M.I.) Descriptions of Pathogenic Fungi and Bacteria. 1970. No. 256. *Verticillium dahliae*. Commonwealth Agricultural Bureaux/Commonwealth Mycological Institute, Kew, Surrey. England. The Eastern Press Ltd., London and Reading, Great Britain. As provided in MRID 46461303.

Smith, I.M., J. Dunez, D. H. Phillips, R.A. Lelliott, and S.A. Archer. Publ. Date unknown. Basic description of *Verticillium dahliae* in European Handbook of Plant Diseases. Pages 299–302. As provided in MRID 46461303.

CLASSIFICATION: The rationale is acceptable to waive non-target plant toxicity/pathogenicity studies, at this time.

STUDY TYPE: Non-target Insect Studies, (OPPTS 885.4340; Gdln 154-23)(MRIDs 45720704; 46461303)

ACTIVE INGREDIENTS: *Verticillium* Isolate 850

SYNONYMS: Dutch Trig™, *Verticillium albo-atrum* WCS 850, *Verticillium dahliae* WCS 850

REQUESTED BY: Arcadis, Inc. and USDA/IR-4

SUMMARY: The request to waive non-target insect studies (OPPTS 885.4340) is based on the rationale that there are no published reports of toxicity or pathogenicity of the MPCA to insects; and the vast majority of these organisms will not be exposed to the MPCA, since intended applications are restricted to injections into elm trees. Furthermore, once applied, *Verticillium* appears to be restricted to the annual ring of the elm, and cannot be isolated from elm trees one year after treatment (MRID 457207-04).

WAIVER REQUEST JUSTIFICATION:

The waiver request is based on the following rationale:

The intended application method of direct injection of *Verticillium* Isolate 850 involves a special injector apparatus ("gouge pistol"), a closed system in which the *Verticillium* conidia (spores) are injected into the trunk of an elm tree (3 drops every 3 or 4 inches around the tree trunk's circumference). The *Verticillium* spores are contained in a small glass vial, which is

DRAFT 24 August 2005

never opened; the vials are “loaded” into the pistol (MRID 457207-04). Because of the “under pressure” in a tree’s xylem tissue, the conidia (suspended in water) are sucked up immediately. Used vials, sealed by a septum, are capped and returned to ARCADIS. The injected MPCA is contained in the annual ring (MRID # 457207-04, p 19 and 45); furthermore, vascular infection appears contained in treated trees (p. 19 and 45, MRID # 464613-03).

CLASSIFICATION: The rationale is acceptable to waive non-target insect toxicity/pathogenicity studies.

NO REFERENCES FROM THE OPEN LITERATURE CITED

STUDY TYPE: Honeybee Testing, (USEPA OPPTS 875.4380; Gdln 154-24)(MRIDs 45720704; 46461303)

ACTIVE INGREDIENTS: *Verticillium* Isolate 850

SYNONYMS: Dutch Trig™, *Verticillium albo-atrum* WCS 850, *Verticillium dahliae* WCS 850

REQUESTED BY: Arcadis, Inc. and USDA/IR-4

SUMMARY: The request to waive non-target honeybee testing, (USEPA OPPTS 875.4380) is based on the rationale that there are no published reports of toxicity or pathogenicity to honeybees, and that these organisms will not be exposed to the MPCA, since intended applications are restricted to injections into elm trees. Furthermore, once applied, *Verticillium* appears to be restricted to the annual ring of the elm, and cannot be isolated from elm trees one year after treatment (MRID 457207-04).

WAIVER REQUEST JUSTIFICATION:

The waiver request is based on the following rationale:

The intended application method of direct injection of *Verticillium* Isolate 850 involves a special injector apparatus (“gouge pistol”), a closed system in which the *Verticillium* conidia (spores) are injected into the trunk of an elm tree (3 drops every 3 or 4 inches around the tree trunk’s circumference). The *Verticillium* spores are contained in a small glass vial, which is never opened; the vials are “loaded” into the pistol (MRID 457207-04). Because of the “under pressure” in a tree’s xylem tissue, the conidia (suspended in water) are sucked up immediately. Used vials, sealed by a septum, are capped and returned to ARCADIS. The injected MPCA is contained in the annual ring (MRID # 457207-04, p 19 and 45); furthermore, vascular infection appears contained in treated trees (p. 19 and 45, MRID # 464613-03).

CLASSIFICATION: The rationale is acceptable to waive non-target insect toxicity/pathogenicity studies.

NO REFERENCES FROM THE OPEN LITERATURE CITED



13544

R151465

Chemical: Verticillium dahliae isolate WCS 850

PC Code:

081305

HED File Code: 41300 BPPD Eco Effects

Memo Date: 8/24/2005

File ID: DPD314718

Accession #: 000-00-9003

HED Records Reference Center

9/21/2007